

Wright (J. H.)

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*On the Cultivation of the Gonococcus from  
Cases of Gonorrhœa, Ophthalmia  
Purulenta, and Pyosalpinx.*

BY

J. H. WRIGHT, A.M., M.D.,  
ASSISTANT IN PATHOLOGY.

*(From the Scars Pathological Laboratory of the Harvard Medical School.)*

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ON THE CULTIVATION OF THE GONOCOCCUS FROM CASES  
OF GONORRHOEA, OPHTHALMIA PURULENTA,  
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THE occurrence of a peculiar diplococcus in the pus of gonorrhœa and ophthalmia purulenta was first pointed out by Neisser<sup>1</sup> in 1879, and named by him the gonococcus. Since that time a great many investigators have studied this organism and its occurrence in various inflammatory processes. The results of this work have fully confirmed the opinion of Neisser, that the gonococcus is the cause of gonorrhœa and ophthalmia purulenta, and have further shown that it is to be found in the vulvo-vaginitis of children, in a certain proportion of cases of pyosalpinx and in some other inflammatory conditions of the genital organs of the female, as well as in some of the cases of endocarditis and arthritis secondary to gonorrhœa in the male. On account of the fact that the ordinary culture media are not suitable for the cultivation of the gonococcus, and because of the difficulty of preparing the special media required for its growth, most of this work has consisted solely in the microscopical study of stained preparations, the recognition and identification of the organism being rendered possible by its morphology, staining reactions, and position inside of the leucocytes.

While the positive identification of the gonococcus can probably be made by a practised observer, in most cases, from these considerations, yet as a method of investigation it is inadequate for at least two reasons: first, because other cocci which resemble it may be mistaken for it; and, second, because it may be present, but escape observation.\*

To confirm and supplement the results obtained by the microscope, the need of an easily prepared culture medium, upon which the gonococcus will readily grow, has long been felt, for by the aid of culture methods the presence or absence of this organism in pathological conditions and its identity might be determined with the same degree of accuracy as is done in the case of other bacteria.

The cultivation of the gonococcus has been attempted by a number of bacteriologists, few of whom have succeeded in obtaining undoubted cultures of it. The first to cultivate it was Bumm,<sup>2</sup> who obtained cultures on coagulated human blood-serum from the pus of gonorrhœa



and ophthalmia purulenta. This investigator was also the first to produce a gonorrhœal urethritis by inoculation from a pure culture, and thus to prove beyond question the etiological significance of the gonococcus.

Wertheim<sup>3</sup> improved the culture method of Bumm by using a mixture of nutrient agar and human blood-serum, upon which the gonococcus was found to grow more vigorously than on the blood-serum alone. The blood-serum was obtained under aseptic precautions, and mixed with melted agar at a temperature of 40° C. In this way he was enabled to prepare plate cultures, and thus to study easily the appearances of the colonies of the organism. It was cultivated by Wertheim from the pus of seven cases of pyosalpinx, two cases of ovarian abscess, and one case of peritonitis secondary to pyosalpinx. The identity of the coccus thus isolated was proven by the production of characteristic urethritis from the inoculation of the cultures from three of the cases. It also grew on media prepared with ox blood-serum, but the growth was less vigorous than on the media containing the human serum.

By the use of the method of Wertheim, Gebhard,<sup>4</sup> Risso,<sup>5</sup> and Menge<sup>6</sup> have also cultivated the gonococcus from gonorrhœal processes. Steinschneider<sup>7</sup> modified Wertheim's media by the addition of sterile urine to the agar and blood-serum mixture. Sterile human serum was used, which was obtained by venesection under aseptic precautions. The organism was cultivated from the pus of gonorrhœa, and its virulence demonstrated on the human urethra.

In all these media, blood-serum, preferably human blood-serum, free from bacteria, is an essential constituent. This fact has hitherto prevented the general use of any of them in the study of infections with the gonococcus, because of the great difficulties, not only of obtaining human blood in sufficient quantities, but also of obtaining the serum of either human or animal blood in sterile condition. Still more impracticable is the use of the plover's egg albumen of v. Schrötter and Winkler,<sup>8</sup> the knee-joint effusion of Aufuso,<sup>9</sup> and the ovarian-cyst fluid of Menge,<sup>10</sup> by means of which the writers named have cultivated the gonococcus. It has also been stated that a slight growth on plain agar and on glycerin-agar has been observed by Wertheim,<sup>3</sup> and this observation confirmed by Günther<sup>11</sup> for the latter medium. These observations seem, however, to be exceptional.

Following in general the method of Steinschneider, during the first six months of 1894 the writer has cultivated from seven cases of gonorrhœal urethritis, eight cases of ophthalmia purulenta, four cases of pyosalpinx, and from one case of vaginitis in a child, a coccus which is in all respects

\* In many cases the staphylococcus pyogenes may occur in purulent collections in the form of diplococci enclosed in cells, and, while not agreeing in all respects with the gonococcus, may closely simulate it. Streptococci may also appear in the same way. In some cases so few organisms may be present that repeated cover-slip examinations may fail to reveal them.



identical with the gonococcus in its morphology, staining reactions, and cultural peculiarities. Although the effect of the inoculation of the human urethra with this coccus has not been tried, yet the organism is held to be the gonococcus for the following reasons:

First. The microscopical examination of the pus, made in most of the cases from which cultures were obtained, showed the presence of typical gonococci inside of leucocytes, and as the only or prevailing organism present.

Second. The growth, on the special media, from such pus, of colonies unlike the colonies of other pyogenic cocci; these, on microscopical examination, were seen to be made up of diplococci like gonococci in their morphology and mode of division.

Third. The fact that these cocci were decolorized by Gram's method of staining, which is a very characteristic peculiarity of the gonococcus.

Fourth. The failure, as a rule, of this coccus to grow on glycerin-agar when transplanted on to this medium from colonies which developed on the special medium; the gonococcus, according to the best authorities, either not growing at all or but to a very slight extent upon agar.

Fifth. The occurrence of one and the same coccus in all of a considerable number of cases of gonorrhœal infection.\*

METHODS. The culture medium used was essentially that of Steinschneider, consisting of a mixture of urine, blood-serum, and nutrient agar. This mixture was contained in test-tubes in the form of "slants." The method of preparation of the medium, however, differs from Steinschneider's in that, instead of collecting the blood-serum and urine under aseptic precautions and preventing their contamination, these constituents were collected without any special care whatever, and were freed from bacteria by filtration through unglazed porcelain. This filtration has been found to be perfectly feasible, and greatly lessens the amount of labor and skill required in preparing this culture material. Both human and ox-blood blood-serum have been employed, the latter evidently serving quite as well as the former. The details of the method are as follows, although they have been departed from at times in non-essential particulars:

A litre of nutrient agar (beef infusion) is prepared in the usual

\* Since this paper was written Turro has reported in the *Centralblatt für Bakteriologie und Parasitenkunde* for July 2, 1894, the cultivation of the gonococcus on acid gelatin. The writer has not been able to confirm this.

No growth has been observed in the acid gelatin prepared according to Turro's directions and inoculated from colonies of the coccus here described. The same negative result has followed the inoculation of the organisms into agar rendered slightly acid by hydrochloric acid. In this connection it may be stated that Dr. William Royal Stokes, Resident Assistant Pathologist of the Boston City Hospital, has recently succeeded in isolating from gonorrhœal urethritis the same coccus described in this paper by the use of the coagulated blood-serum mixture of Loeffler rendered slightly acid. This medium is coagulated by dry heat in test-tubes in the form of "slants," and sterilized by steam, as in the case of ordinary culture media.

manner, and after filtration is evaporated to about 600 cc. This concentration is desirable, so that, after the dilution with the urine and serum, the medium may be sufficiently firm. This concentrated agar is then run into test-tubes, and the whole sterilized by steam three times. The quantity of agar placed in each tube is smaller than is usual, in order to allow for the subsequent addition of the urine and serum.

The blood-serum, which need not be free from corpuscles, is first passed through white sand, which is supported in a funnel by filter-paper, in order to remove as much as possible any particles in suspension, and is then mixed with half its volume of fresh urine. The mixture of urine and blood-serum is next filtered by suction through an unglazed porcelain cylinder into a receiving flask, such as chemists use for similar purposes, by means of a water-vacuum pump.

This frees the mixture from bacteria.

The usual precautions are, of course, taken to prevent the contamination of the filtrate, such as the previous sterilization by steam of the cylinder and receiving flask, besides others which will occur to any bacteriologist.

The greatest difficulty which has been encountered has been the slowness of the filtration through the porcelain. This has been very satisfactorily overcome, however, by the use of a cylinder manufactured by the Boston Filter Company, through which the mixture of urine and serum passes rapidly.

To the agar in each test-tube, which is fluid and of a temperature of about 40° C., there is added about one-third to one-half its volume of the filtered mixture of urine and blood-serum. This is conveniently accomplished by pouring the mixture from the receiving flask through the lateral tube, inserted near its neck, directly into the tubes. The melting of the agar is best effected in the steam sterilizer in order that any organisms which have found lodgment in the cotton plugs of the tubes may be destroyed. When the agar is melted it is cooled and kept fluid by placing the tubes in a water-bath at 40° C. Each tube, after the addition of the urine and serum to the fluid agar, is quickly shaken to assure a good admixture, and is then placed in a slanting position to allow the agar to become solid, and thus to form a slant. When the medium in the tubes has solidified the tubes are placed in the incubator for about twenty-four hours to test for contaminations, after which they are ready for use.

Over the surface of the culture medium thus prepared a small quantity of the pus is distributed and the culture placed in the incubator for development. At the same time a stained cover-slip preparation of the pus was examined in most of the cases. In some cases the material for examination has been secured and brought to the laboratory on a "swab," which consists of a piece of absorbent cotton wound on the end



of a piece of stiff wire. This is carried about in a test-tube in which it has previously been sterilized by dry heat.

**BIOLOGICAL CHARACTERS OF THE GONOCOCCUS.** After twenty-four hours in the incubator the colonies of the gonococcus appear on the urine-serum-agar as grayish, semi-translucent points, a fraction of a millimeter in diameter.

After forty-eight hours they may be about one millimeter or so in diameter, slightly elevated, with a rounded outline, grayish in color, and by transmitted light semi-translucent. By reflected light their surface has the appearance of frosted glass. Later, if few in number, so that their growth is unimpeded, the colonies may attain a diameter of two millimeters or more, become thicker and denser, with a faintly-brownish tinge about their centres and a slightly irregular outline.

Under a low power of the microscope a fully-developed colony is seen to consist of a generally circular expansion, with thin, translucent, smooth, sharply-defined margin, but becoming brownish, granular, and thicker toward the central portion, which is made up of coarse, granular, brown-colored clumps closely packed together.

The appearances coincide with the figure of such a colony given by Wertheim.<sup>3</sup>

In daughter cultures the colonies become confluent along the line of inoculation on the surface of the medium. As has been pointed out above, one of the important characteristics of the gonococcus is its inability to grow on nutrient agar, although some observers have noted a minimal development on this medium.

In the present work the growth, on glycerin-agar, of the coccus here described has been tested by transplantation from the colonies in a majority of the cases in which it has been cultivated. With two or three exceptions, in none of these cases was any development on the agar obtained. In the exceptional cases the growth which appeared was very scanty.

When transplanted onto coagulated ox-blood-serum (Löffler's mixture sterilized by steam) the organism grows in the form of minute colonies. This medium is not, however, to be regarded as suitable for its cultivation.

In morphology the coccus which has been cultivated from the cases detailed below is identical with Neisser's organism.

Under the microscope, in stained preparations from cultures, it appears as a diplococcus, somewhat larger than the other pyogenic cocci—composed of two hemispheres separated by a narrow unstained interval. Frequently two of these hemispheres are seen lying side by side in the manner of tetrads, and showing clear evidence that division occurs in two directions at right angles to each other.

As was first shown by Roux,<sup>12</sup> an important characteristic of the gonococcus, which has been alluded to above, is the fact that it is decol-

orized when treated by Gram's method of staining. In this it differs strikingly from other pyogenic cocci. The coccus here described has been found to lose its color in every instance in which it has been treated according to Gram's method, and this has been done in every case in which it has been cultivated.

The duration of life of the organism in cultures seems to be variable, apparently depending largely upon the quality of the culture medium used.

Although no extensive studies have been made of this point in the present work, yet, in general, it may be said that it seems to lose its vitality in cultures more rapidly than do the other pyogenic cocci, growth from a colony a week old or even less being uncertain. Wertheim, however, notes that a culture more than six weeks old retained its vitality, while Bumm found that the ability to grow in daughter cultures was lost after a few days.

GONORRHOEA. Most of the cultures from gonorrhœal urethritis have been secured through the coöperation of Dr. Abner Post, of Boston, at whose suggestion the experiments in the cultivation of the gonococcus were undertaken.

The writer is under many obligations to Dr. Post for the active interest which he has shown in the work. Of other cultures one was obtained from a case in the practice of Dr. W. H. Prescott.

From the pus of seven cases of acute gonorrhœal urethritis in the adult male characteristic colonies have been cultivated, which on microscopical examination were found to consist of cocci identical with gonococci in their morphology and decolorizing by Gram's method of staining.

The microscopical examination of the pus made in five of these cases showed the presence of the gonococcus in each as the prevailing or only coccus present. The colonies in five of the cases were numerous, being thickly crowded together over the surface of the medium. In one case they appeared in moderate numbers, and in another case but three colonies developed. In this last case very few gonococci were to be seen in the cover-slip preparation of the pus.

Other bacteria have also developed in these cultures, but their colonies are usually much smaller in number or develop more slowly than those of the specific cocci, so that they have caused but little annoyance.

Only cases of gonorrhœa of recent origin seem to be suitable for obtaining cultures of the gonococcus. Cases which have persisted for a considerable time are unfavorable for the growth of discrete colonies of the organism, because of the comparatively great number of rapidly-growing bacteria of various kinds which overgrow the surface of the medium.

It is worthy of note that one of the cases from which numerous colonies developed was certainly acquired through active pæderasty.



In addition to these cases of urethritis, through the kindness of Dr. James M. Jackson, a culture has been obtained from a case of vaginitis in a child of nine years of age. There had been a purulent discharge from the vulva during about a week before being seen by Dr. Jackson, who found the vulva inflamed and red, hymen injected but intact, and on opening the vagina a considerable amount of yellow pus exuding. No history of contagion or *stuprum*.

Microscopical examination of the pus showed numerous cocci resembling gonococci as the prevailing organism, only a few of which were seen inside of the leucocytes.

A small amount of the pus was spread over the surface of the urine-serum-agar "slant." On this, after twenty-four hours in the incubator, very numerous minute grayish, semi-translucent colonies appeared. These, on microscopical examination, were found to be composed for the greater part of cocci resembling gonococci in morphology, and apparently undergoing division in two directions. They were decolorized by Gram's method of staining. A few pneumococci-like bacteria were also present, but in inferior numbers.

From the culture two glycerin-agar tubes were inoculated. After some days in the incubator no growth at all could be seen in one of the tubes, while in the other it was doubtful from the appearances whether there had been any growth or not.

OPHTHALMIA PURULENTA. The gonococcus was first cultivated from this disease by Bumm,<sup>2</sup> and since, Gebhard<sup>4</sup> and Menge<sup>6</sup> have each cultivated it from single cases. In the present work it has been cultivated from eight cases.

Through the kindness of Dr. Henry H. Haskell, Resident Ophthalmologist of the Massachusetts Charitable Eye and Ear Infirmary, cultures have been obtained from five cases of ophthalmia. The writer is indebted to Dr. Haskell for some of the clinical notes on these cases:

CASE I.—Child, aged six years, entered Massachusetts Charitable Eye and Ear Infirmary with history of sore eyes for about two weeks. Lids œdematous, profuse purulent discharge. Conjunctivæ of lids and globe much injected and chemosed. Cornea clear and not affected. Steady improvement under treatment. Clinically the case was doubtfully specific. Source of infection unknown. After two weeks discharge had ceased, no œdema of lids, no chemosis of conjunctivæ, globe white. Discharged.

On the seventh day after admission a culture was taken from the pus, and a moderate number of minute grayish semi-translucent colonies developed on the *urine-serum-agar*. These were found to be made up of cocci like gonococci in morphology and mode of division, and were decolorized by Gram's method of staining.

No growth from transplantation on glycerin-agar after some time in incubator.

A second culture was taken two days later, in which rather numerous colonies of the same coccus developed. In the appearance of its colonies, in its morphology, mode of division, and staining peculiarity, it was identical with the coccus which grew in the first culture.

No growth when transplanted onto glycerin-agar after three days in incubator.

No organisms were found in a cover-slip preparation of the pus of the second culture, stained by Gram's method, as was to be expected from the fact that the gonococci were decolorized, if present.

If ordinary pus organisms had been present, they would have been stained, and therefore rendered visible.

A third culture was made, after another day, from the cloudy, watery discharge which was then occurring. Microscopical examination of this pus in a preparation stained in the ordinary way showed leucocytes and cocci like gonococci. No typical phagocytosis was seen. A few colonies of the same appearance, and made up of the same coccus as in the other cultures, developed on the surface of the culture tube. This coccus was like the gonococcus in morphology, mode of division, and reaction toward Gram's method of staining.

Transplanted onto glycerin-agar it failed to grow.

CASE II.—Boy, aged ten years, entered the Massachusetts Charitable Eye and Ear Infirmary with a history of sore eyes for ten days. Purulent discharge, not abundant. Lids oedematous, conjunctivæ of lids and globe much chemosed and injected. Corneæ not affected. Steady improvement under treatment.

Discharged after about a week with eyes in good condition. Clinically the nature of this case was doubtful. Source of infection unknown.

On both the third and fifth days after entrance a culture was made from the pus.

In each tube numerous characteristic colonies of cocci developed, together with a few colonies of other bacteria in one of the tubes. The coccus grown on each occasion was like the gonococcus in morphology, mode of division, and reaction toward Gram's method of staining. Transplanted onto glycerin-agar there was no development in the case of one of the cultures, and in the other there grew two small colonies of the coccus.

A cover-slip preparation of the pus in this case showed typical gonococci, which were decolorized by Gram's method of staining.

CASE III.—Female, aged twenty-five years, married. Entered the Massachusetts Charitable Eye and Ear Infirmary with history of sore eye for about a week previously. Only one eye affected. Oedema of lids. Conjunctivæ of lids and globe much chemosed. Cornea perforated. Abundant purulent discharge. Infection from husband almost certain.

Two days after admission a culture was made from the pus, and at the same time a cover-slip preparation. The cover-slip preparation, stained in the ordinary manner, showed a few typical gonococci in leucocytes.

In the culture numerous characteristic colonies developed, along with a few other colonies.

The numerous colonies were composed of cocci like gonococci in their morphology, mode of division, and reaction toward Gram's method of staining, which did not grow when transplanted onto glycerin-agar.

CASE IV.—Infant. Massachusetts Charitable Eye and Ear Infirmary.



Thick, purulent discharge from eyes; cover-slip preparation of the pus stained in the usual manner showed typical gonococci in leucocytes. No other bacteria noted as present. Stained by Gram's method the gonococci were seen to be decolorized.

In the culture made from the pus there grew very numerous characteristic colonies of cocci like gonococci in their morphology, mode of division, and reaction toward Gram's method of staining.

The coccus did not grow when transplanted onto glycerin-agar and kept in the incubator for some days.

CASE V.—Infant. Massachusetts Charitable Eye and Ear Infirmary. Purulent discharge from eyes. A culture from the pus developed numerous characteristic colonies of cocci, which were like gonococci in their morphology, mode of division, and reaction toward Gram's method of staining.

In a transplantation onto glycerin-agar, after forty-eight hours in the incubator, a very slight growth was noted. A few other colonies also grew from the pus.

CASE VI.—St. Elizabeth Hospital. The writer is indebted to Dr. James M. Jackson for this case and its history.

Female, aged fourteen years, abundant discharge of thick, creamy pus from one eye. Duration of disease but a few days at the time the material for cultures was secured, although there was a history of a sore eye for a month. Infant in family said to have had ophthalmia.

In the culture from the pus there developed numerous characteristic colonies of cocci, which were like gonococci in their morphology, mode of division, and reaction toward Gram's method of staining. They did not grow when transplanted onto glycerin-agar which was kept three days in the incubator.

No microscopical examination of the pus was made.

CASE VII.—Boston City Hospital. Infant, discharge from eyes of thick, yellow pus. A cover-slip preparation of the pus, stained in the usual manner, showed the presence of gonococci.

In a culture from the pus there developed about fifteen characteristic colonies of cocci, which were like gonococci in their morphology, mode of division, and reaction toward Gram's method of staining.

But one colony of another organism developed.

There was no growth of these cocci transplanted onto glycerin-agar after they had been forty-eight hours in the incubator.

CASE VIII.—Boston City Hospital. Infant, purulent discharge from eyes.

But few gonococci were found in the pus by microscopical examination. In the culture from the pus only two colonies developed. These seemed to be gonococci on microscopical examination, and were decolorized by Gram's method of staining. It is uncertain whether they grew on glycerin-agar or not.

PYOSALPINX.—The close etiological connection between certain of the inflammatory conditions of the pelvic organs of the female and gonorrhœa was first clearly pointed out by Noeggerath,<sup>13</sup> on clinical grounds alone, in 1872, and has been generally admitted for some time.

In pyosalpinx, gonococci were first observed with the microscope by Westermarck,<sup>14</sup> in 1886.

By the microscopical examination alone they have since been found in salpingitis by Orthmann<sup>15</sup> in one of eight cases, by Schmitt<sup>16</sup> in one case, by Steman<sup>17</sup> in one case, by Menge<sup>18</sup> in three of twenty-six cases, by Zweifel<sup>19</sup> in eight of forty-four cases, by Reichel<sup>20</sup> in one case, by Döderlein<sup>21</sup> in one case, and by Wertheim<sup>3</sup> in nine of twenty-four cases. In addition to these cases, Wertheim<sup>3</sup> has isolated it in cultures from seven cases, and Menge<sup>10</sup> from one case also. In all, about one hundred and eight cases have been recorded in the literature, which have been examined for the presence of gonococci, either by the microscope or by cultures, and in thirty-four cases, or 31 per cent., this organism has been found. Sixty-one of the cases were sterile or free from bacteria, and in but eleven of the one hundred and six cases were other bacteria than gonococci present.

In the present work gonococci have been demonstrated by cultures in four out of about twenty cases of pyosalpinx, or in about 20 per cent. Excluding one specimen, which had been in communication with the sinus in the abdominal wall, and another, in which there was tuberculosis, the contents of all the negative tubes were free from bacteria, both by cultures and by microscopical examination.

This result is in harmony with the experience of others referred to above. A large part of the material for study has been obtained through the kindness of Dr. James M. Jackson of the staff of St. Elizabeth Hospital, for which the writer desires here to make acknowledgment. The cases of pyosalpinx from which gonococci were cultivated are as follows:

CASE I.—Operation at St. Elizabeth Hospital, specimen brought to laboratory by Dr. Jackson. Tube enlarged and containing a considerable amount of thin pus. Cover-slip preparations of the pus showed the presence of gonococci, not numerous, but easily found. On the surface of the culture made from the pus there developed probably sixty characteristic colonies which were made up of cocci like gonococci in their morphology, mode of division, and reaction toward Gram's method of staining.

CASE II.—Operation at St. Elizabeth Hospital, specimens brought to laboratory by Dr. Jackson. One tube very much enlarged and tortuous and distended with pus. The other tube is smaller and also contains pus. Cover-slip preparations of the pus from the larger tube were negative for bacteria, but in one from the smaller tube one or two leucocytes containing gonococci were seen.

Four or five cultures were taken from the contents of these tubes, in all of which a moderate number of characteristic colonies developed. Transplantations from these colonies into four glycerin-agar tubes resulted in no growth.

CASE III.—Operation by Dr. Burrage at the Carney Hospital. Both tubes contained pus. In the pus of one of them gonococci were seen by the microscope. In a culture made from the pus of one of them three or four characteristic colonies and one or two fluorescent colonies developed.



The characteristic colonies were composed of cocci, like gonococci in morphology, which were decolorized by Gram's method of staining.

CASE IV.—Operation by Dr. Mixter. The walls of the tubes were somewhat thickened and there was a small amount of pus in the interior. In a culture made from the pus a moderate number of characteristic colonies of cocci developed. These cocci were like gonococci in their morphology, mode of division, and reaction toward Gram's method of staining.

No growth in a transplantation onto the glycerin-agar after forty-eight hours in the incubator.

A cover-slip preparation from the interior of the tube made after the specimen had been about one day in Müller's fluid was negative for bacteria.

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